PennVet | CREATE
University of Pennsylvania
Carbapenem Resistant Enterobacterales Animal Testing and Epidemiology

mCIM

Required supplies	Recommended products/Vendor
Tryptic Soy Broth	R07222/Fisher scientific
1 uL and 10 uL Loops	
Meropenem Sensi-disc	B4331703/Fisher scientific
ATCC 25922 Escherichia coli	R4607050/Fisher scientific
Mueller Hinton agar	R01624/Fisher scientific
Saline	

QC strains

- Positive control: ATCC BAA-1705 Klebsiella pneumoniae
- Positive control: ATCC BAA-2146 Klebsiella pneumoniae (also eCIM positive)
- Negative control: ATCC BAA-1706 Klebsiella pneumoniae

Procedure

1. Add a Meropenem(MEM) Sensi-disc to 2 mL of Tryptic Soy Broth(TSB)
2. Inoculate 1 uL loopful of suspect Enterobacterale (or 10 uL loopful Ps. aeruginosa) into the TSB w/MEM
3. Vortex
4. Incubate at 35° C ±2° C for 4 hours
5. After 4 hours, prepare a McFarland Standard of ATCC 25922 E. coli and streak a lawn onto a Mueller Hinton agar plate
6. Remove the MEM disc from the inoculated TSB, making sure to remove excess liquid from the disc by dragging on the side of the tube. Place in the center of the inoculated Mueller Hinton agar. Discard the TSB
    a. It is helpful to invert the TSB tube and let the MEM disc fall to the top. Then slowly return the tube to the upright position, careful to make sure the MEM disc stays at the top. Then the MEM can be more easily removed from the tube using a 10 uL loop
7. Incubate the Mueller Hinton agar w/ MEM for 18-24 hours at 35° C ±2° C
8. After 18-24 hours, measure the zone of inhibition surrounding the MEM disc in mm

Result interpretation guide

Positive	Interpretation
<16 mm	Positive
≥19mm	Negative
16-18mm	Indeterminate

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eCIM

Required supplies	Recommended products/Vendor
Tryptic Soy Broth	R07222/Fisher scientific
10 uL Loops	
Meropenem Sensi-disc	B4331703/Fisher scientific
ATCC 25922 Escherichia coli	R4607050/Fisher scientific
Mueller Hinton agar	R01624/Fisher scientific
Saline	
0.5 M EDTA	

QC strain

- Positive control: ATCC BAA-2146 Klebsiella pneumoniae (also mCIM positive)
- Negative control: ATCC BAA-1706 Klebsiella pneumoniae

Procedure

1. Add 20 uL of 0.5 M EDTA to 2 mL Tryptic Soy Broth (TSB)
2. Add a Meropenem disc (MEM) to the TSB w/EDTA
3. Inoculate 1 uL loopful of suspect Enterobacterale (or 10 uL loopful Ps. aeruginosa) and mix thoroughly
4. Vortex
5. Incubate at 35° C ±2° C for 4 hours
6. After 4 hours, prepare a McFarland Standard of ATCC 25922 E. coli and streak a lawn onto a Mueller Hinton agar plate
7. Remove the MEM disc from the inoculated TSB, making sure to remove excess liquid from the disc by dragging on the side of the tube Place in the center of the inoculated Mueller Hinton agar. Discard the TSB
    a. It is helpful to invert the TSB tube and let the MEM disc fall to the top. Then slowly return the tube to the upright position, careful to make sure the MEM disc stays at the top. Then the MEM can be more easily removed out from the top of the tube using a 10 uL loop
8. Incubate the Mueller Hinton agar w/ MEM for 18-24 hours at 35° C ±2° C
9. After 18-24 hours, measure the zone of inhibition surrounding the MEM disc in mm

Result interpretation guide

Positive	Interpretation
≥5 mm increase in zone diameter compared to the mCIM	Positive
≤ 4 mm increase in zone diameter compared to the mCIM	Negative

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CarbaNP

Required supplies
Microcentrifuge tubes
Bacterial protein extraction reagent in Tris HCl buffer, pH 7.4
1 uL loops
10 mM zinc sulfate heptahydrate solution
0.5% phenol red solution
0.1 N sodium hydroxide solution
Imipenem powder
CarbaNP solution A (see below)
CarbaNP solution B (solution A + imipenem, see below)

QC strains

- Positive control: ATCC BAA-1705 Klebsiella pneumoniae
- Negative control: ATCC BAA-1706 Klebsiella pneumoniae

CarbaNP Solution A preparation

1. Add 2 mL of 0.5% phenol red solution to 16.6 mL reagent water
2. Add 180 uL of 10 mM zinc sulfate solution
3. Adjust the pH using 0.1 N NaOH solution (if pH is too low) or 10% HCl (if pH is too high) to reach a final pH of 7.8 ± 0.1
4. Store at 4-8° C

CarbaNP Solution B preparation

1. Add 6 mg of imipenem powder per 1 mL CarbaNP Solution A
2. Store at 4-8° C up to 3 days

Procedure

1. Label one microcentrifuge tube “A” and one microcentrifuge tube “B” for each isolate plus an uninoculated control
2. Add 100 uL of bacterial protein extraction reagent to each tube
3. Inoculate 1uL loopful of bacteria from a blood agar plate into both A and B tubes. Mix thoroughly
4. Vortex for 5 seconds
5. Add 100 uL of solution A into tube A
6. Add 100 uL of solution B into tube B
7. Vortex
8. Incubate at 35° C ±2° C for up to 2 hours

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Result interpretation guide

Tube A	Tube B	Interpretation
Red or red-orange	Red or red-orange	Negative
Red or red-orange	Light orange, dark yellow or yellow	Positive
Red or red-orange	Orange	Indeterminate
Orange, light orange, dark yellow or yellow	Any color	Indeterminate